IN VITRO PREDICTION OF SUNSCREEN PFA VALUES

RELATED APPLICATIONS

This patent application claims priority to Provisional Patent

5   Application Serial No. 60/447,092 filed February 13, 2003 and Provisional

Patent Application Serial No. 60/506,833 filed on September 29, 2003, with

both applications being incorporated by reference herein.

BACKGROUND OF THE INVENTION

10

1.   Field of the Invention

The invention relates generally to the *in vitro* prediction of a

composition's sunscreen PFA values. More particularly, the present

invention relates to an *in vitro* method for the prediction of *in vivo* UVA

15   protection by a composition with sunscreen properties.

2.   Description of Related Art

It is known that the UV portion of sunlight causes skin damage.

UVB irradiation is almost entirely absorbed by the epidermis and it causes

20   an erythemal or sunburn reaction, as well as induces DNA mutations.

UVA irradiation is capable of reaching dermal layers and even

affecting circulating blood cells. Compared with UVB, UVA generates more

oxidative stress, which induces matrix metalloproteases and suppresses

25   skin immune function.

Sunscreen compositions or any compositions with sunscreen active material are designed to protect skin from photodamage that may result from UV light exposure. It is becoming more important to develop sunscreens that effectively protect from both UVB and UVA.

5

UVB protection of a sunscreen is measured by a FDA approved SPF *in vivo* method that utilizes erythema as a biological endpoint. In contrast to the SPF method there is no official method to measure photoprotection against UVA. At the same time, effective protection against the UVA

10   portion of the solar spectrum associated with cumulative skin damage is an important element of sunscreen and anti-aging cosmetic formulations.

The JCIA (Japan Cosmetic Industry Association) method is an *in vivo* method that is the most often used in the U.S. According to this

15   method, Protection Factor A (PFA) based on persistent pigment darkening (PPD) can determine UVA protection provided by sunscreens. It was adopted by JCIA in 1995 as an official method. Additional methods modeled after the JCIA method are described in *The Reproducibility of an In-Vitro Determination of the UVA INDEX Describing the Relative UVA*

20   *Protection of Sun Care Products*, Gers-Barlag et al., IFSCC Magazine, Vol. 5, No. 3, 2002; and *A New In-Vitro Test Method to Assess the UVA Protection Performance of Sun Care Products*, V. Wendel et al., SOFW 2001, 127.

Since PPD is produced in the basal keratinocytes by a photochemical conversion of preexisting melanin and its precursors and/or migration of melanosomes, it may be assumed that PPD gives a direct estimate of the UVA impact on the viable epidermis. PPD is a stable skin response that is linearly dependent on the amount of UVA that enters the viable epidermis. In the JCIA method, the PFA is determined from the ratio of the sunscreen-protected minimal PPD to the unprotected PPD, evaluated 2 to 4 hours after UVA exposure. This method utilizes a xenon arc light simulator filtered with a 2 millimeter (mm) WG335 and 1mm UG11 filters and volunteers with skin types II, III and IV.

Time of UVA exposure during *in vivo* PFA testing of a sunscreen is based, according to the test protocol, on its PFA estimated value that was determined *in vitro* prior to the human tests. Thus, correct *in vitro* prediction of the PFA range of the test product is absolutely essential for the accuracy of *in vivo* tests and must be given to the testing lab prior to the study on volunteers. In the prior art methods, *in vitro* UVA protection is calculated using the integration area of normalized spectrum of a sunscreen in entire UVA region (UVA1 + UVA2), which does not result in the accurate prediction of *in vitro* PFA values.

To overcome the deficiencies and inaccuracies associated with the prior art methods, the present invention provides a method for accurately predicting the *in vitro* PFA range for suncare compositions.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide an *in vitro* method for prediction of *in vivo* UVA protection provided by sunscreens.

5   It is another object of the present invention to provide such a method that is reproducible.

It is yet another object of the present invention to provide such a method that correlates well with *in vivo* results.

10

These and other objects are achieved by an *in vitro* method for the prediction of *in vivo* UVA protection by a composition with sunscreen properties. The method includes the steps of: determining *in vivo* SPF; determining *in vitro* SPF based on the sunscreen's absorbance spectrum in

15   the UV region; normalizing (adjusting) the absorbance spectrum of a sunscreen to correspond to its *in vivo* SPF; and calculating the PFA-PPD *in vitro* based on the integration area of normalized absorbance spectrum of a sunscreen in UVA1 region.

20   The terms "suncare composition" and "sunscreen composition" in the context of this application are meant to be interchangeable and include not only sunscreen compositions, but any compositions having one or more sunscreen actives. Other such compositions may include, but are not limited to, cosmetics, insect repellants, lip balms, shampoos, conditioners,

25   gels, topical lotions, and the like.

4

DESCRIPTION OF THE FIGURES

Figure 1 is a graphical representation of the normalization of the SPF measured *in vitro* to that SPF measured *in vivo*;

5 Figure 2 is a graphical representation of a comparison of Commission International de L'Eclairage (CIE) UV Hazard (erythemal) and PPD action spectra;

Figure 3 is a graphical representation of the correlation between *in* 10 *vitro/in vivo* predictions based on both the UVA1 integration area and the UVA1+UVA2 integration area; and

Figure 4 is another graphical representation of the correlation between *in vitro/in vivo* predictions based on both the UVA1 integration 15 area and the UVA1+UVA2 integration area.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides an accurate method for predicting *in vitro* PFA values in sunscreen compositions. The method includes the 20 steps of: determining *in vivo* SPF; determining *in vitro* SPF based on the sunscreen's absorbence spectrum in the UV region; normalizing the absorbence spectrum; and calculating the PFA-PPD *in vitro* based on the integration area of normalized absorbance spectrum of a sunscreen in UVA1 region.

25

To obtain *in-vitro* prediction for PFA-PPD values of a sunscreen composition the method of the present invention, including the steps set forth below, is employed.

5   Step 1. Determine *in-vivo* SPF for test formulation on at least 5 panelists.

The determination is based on the method outlined in the Food and Drug Administration (FDA) Final Monograph for sunscreen testing
10   published in the Federal Register, Vol. 64, No. 98, May 21, 1999, which is incorporated by reference herein.

Step 2. Determine *in-vitro* SPF of sunscreen based on its absorbance spectrum in UV region.
15

This step may be conducted by any suitable method known in the art. By way of example, a suitable method includes, but is not limited to, the method described in *SPF Analysis of Sunscreens*, Technical Note for Labsphere UV-1000S UV Transmittance Analyzer, which is incorporated by
20   reference herein.

The type of substrate and the application dose applied to the substrate have been unexpectedly found to be important factors in the accuracy of *in vitro* measurements of sunscreen absorbance spectra. In
25   addition, it is preferred that the irradiance spectrum of the lamp source

used reflect the irradiance spectrum used in the lamp source used in the *in-vivo* SPF test, as described in Step 1 above.

To achieve the most accurate measurements, the substrate used in
5   the present invention should closely resemble mammalian skin. Therefore, the substrate should be transparent to UV light and simulate the porosity and texture of skin. Suitable substrates for use in the invention may include, but are not limited to, surgical tape such as 3M Transpore™, polyvinyl chloride film such as Saran Wrap®, and synthetic skin substitute
10   material such as Vitro-Skin®. Preferably the substrate used in the present invention is Vitro-Skin®.

Properly selecting the application dose of the sunscreen composition to be applied to the substrate is also important. It has been found that the
15   use of an application dose of 2 mg/cm^2, which corresponds to the application dose used during *in-vivo* PFA (PPD, JCIA) and also during *in vivo* SPF tests, provides the most accurate results in determining *in vitro* PFA.

20   In a preferred embodiment of the present invention, a Labsphere UV-1000S Transmittance Analyzer was used in conjunction with Vitro-Skin® as the substrate and a sunscreen application dose of 2 mg/cm^2.

Step 3. If the *in-vitro* SPF differs from the label SPF, the adjustment
25   of the absorbance spectrum is needed to normalize it to the label value

obtained *in vivo*. Equation 1 is used to normalize the absorbance
spectrum.

Equation I :

$$SPF \text{ in vivo} = SPF \text{ in vitro} = \frac{\int\limits_{290nm}^{400nm} E(\lambda) \cdot S(\lambda)}{\int\limits_{290nm}^{400nm} E(\lambda) \cdot S(\lambda) / 10^{[A(\lambda)\cdot C]}}$$

Where :

$E(\lambda)$ = irradiance at wavelength λ of the light spectrum used

$S(\lambda)$ = effectiveness of a biological endpoint at wavelength λ

      Note : for the SPF it is erythema action spectrum

$A(\lambda)$ = absorbance

C = constant factor for the adjustment of the spectrum

5   This eliminates the impact of the application technique on the
absorbance spectrum of a sunscreen. During this mathematical
adjustment, only the height of the sunscreen's absorbance spectrum is
adjusted by a scalar multiplier in an iterative method to correspond to its *in
vivo* (ACTUAL) SPF value.

10

The convolution spectrum was calculated by multiplying the
irradiation spectrum with CIE UV hazard spectrum. The sunscreen
absorbance spectrum is then incorporated into the convolution spectrum in
order to obtain *in vitro* SPF. The configuration of the spectrum reflecting

15   the specific sunscreen composition remains unchanged. This
"normalization" also takes into the account, at least partially, the

implications of possible photo-instability of the sunscreen during its PFA
study.

5   Figure 1 demonstrates the normalization of the SPF measured *in
vitro* to that SPF measured *in vivo*.

If the *in vitro* SPF is equal to the *in vivo* SPF then no adjustment or
normalization is needed because in this case C=1 and the integration area
of the absorbance spectrum of sunscreen in UVA1 (340-400 nm) region "as
10   is" can be used for *in vitro* estimation of the PPD value.

Step 4. Calculate PFA-PPD *in vitro* using equation 2 set forth below.

Equation 2 :

$$\text{PFA - PPD in vitro} = \frac{\int\limits_{340nm}^{400nm} E(\lambda)\cdot S(\lambda)}{\int\limits_{340nm}^{400nm} E(\lambda)\cdot S(\lambda)/10^{[A(\lambda)c]}}$$

Where :

$E(\lambda)$ = irradiance at wavelength λ of the light spectrum used

$S(\lambda)$ = effectiveness of a biological endpoint at wavelength λ

    Note : for the PPD prediction it is PPD action spectrum

$A(\lambda)$ = absorbance

C = constant factor for the adjustment of the spectrum as in Eq.1.

15   It is preferred that the irradiance spectrum of the lamp source used
reflect the irradiance spectrum used in the lamp source used in the lab PFA
testing.

9

In addition, an important consideration when predicting the PFA values of a sunscreen composition is the effects of UVA2 (320 – 340 nm) and UVA1 (340 – 400 nm) on skin. Careful examination of the effects of UVA on the skin and underlying structures has led to the division of the

5   spectrum into two subsets, namely UVA2 (320 – 340 nm) and UVA1 (340 – 400 nm).

UVA radiation can cause two types of damage depending on the wavelength band. UVB and UVA2 light cause erythema (sunburn) and

10   raise the risk of skin cancer far more than UVA1. UVA1 activates skin enzymes that break down collagen and also activates photoactive molecules in the cell that in turn damage the DNA. UVA1 plays an important role in photoageing, especially wrinkling and sagging of the skin. Recent studies show that UVA2 is responsible for the suppressing

15   established immune reactions. On the other hand, no immune suppression was noted after irradiation with UVA1 only.

As demonstrated in Figure 2, a comparison of CIE UV Hazard (Erythemal) and PPD action spectra also shows the difference between

20   skin biological response plotted against photon energy in UVA1 and UVA2 areas.

It is the UVA1 region of spectrum that produces a tanning (PPD) response. Therefore, since the PFA prediction is based on the PPD, as

25   evident in equation 2 above, the method of the present invention uses only

10

the UVA1 spectrum to measure the PFA. As a result, the prediction of the

PFA values in sunscreen compositions is highly accurate.

In lieu of the above, unexpected findings, in a preferred embodiment

5   of the present invention, when calculating the PFA-PPD$_{in\ vitro}$, the integration

area of "normalized" absorbance spectrum of sunscreen in the UVA1 (340-

400 nm) spectrum is used in conjunction with a xenon arc 150 wt lamp

filtered with 2 mm WG355 and 1mm UG11, and the PPD action spectrum

(Figure 2).

10

In a preferred embodiment, step 2 through step 4 is repeated at

least five times. From the set of calculated *in vitro* PFA-PPD's, the average

and the standard deviation should be calculated. The lower bound

(average minus three standard deviations) should be reported as the

15   measured *in vitro* PFA-PPD.

EXAMPLES

The proposed method has been evaluated in PFA (PPD) studies of

20   fifteen commercially available sunscreens, set forth in Table 1 below.

Tested sunscreens contained various combinations of FDA approved

organic and inorganic sunscreen actives.

25

11

Table 1.

| | | PFA - P P D , J C IA | In vitro Prediction Based on: | |
Product	S P F		U V A 1 & U V A 2	U V A 1
1	5 0	8 . 6 6	1 2 . 0 8	9 . 4 2
2	3 0	9 . 4 9	1 1 . 7 7	9 . 4 1
3	5 0	1 3 . 0 7	1 7 . 5 4	1 3 . 0 3
4	5 0	8 . 3	1 1 . 9	9 . 4
5	3 0	6 . 3 3	9 . 0 5	7 . 8 2
6	3 5	9 . 7 4	1 4 . 7 7	1 2 . 5
7	3 0	8	1 1 . 2	9 . 5
8	5 0	1 2 . 5	1 5 . 2	1 1 . 7 1
9	5 0	5 . 6 6	9 . 8 4	8 . 3 9
1 0	3 0	3 . 1 6	4 . 2	3 . 5
1 1	3 0	3 . 0 4	4	3 . 4
1 2	4 5	1 6 . 3 2	2 1 . 0 9	1 7 . 0 8
1 3	5 0	7 . 0 7	9 . 9 9	8 . 0 2
1 4	4 5	5 . 0 7	7 . 8 5	6 . 1 4
1 5	2 0	5 . 8 2	7 . 8 1	6 . 1 9

In Vitro/In Vivo Data

5   It was found that integration area of 340-400 nm (UVA1) utilized in

the proposed method provides better *in vitro/in vivo* correlation as

compared to the integration area of 320-400 nm (UVA1 and UVA2) and is

aligned with existing knowledge regarding skin biological responses to the

irradiation by UVA1 and UVA2 wavelength bands.

10

As demonstrated in Figure 3, the statistical comparisons of the two

methods with the perfect fit (= PFA measured *in vivo*) indicated that the *in*

vitro method based on integration area of UVA1 only gives a better fit (sum

of squared residuals of 24.97) than the *in vitro* method based on both

15   UVA1 and UVA2 (sum of squared residuals of 162.5).

The method of the present invention has also been evaluated against seven CTFA products, as set forth below in table 2, and has also confirmed that *in vitro* prediction based on integration area of UVA1 only gives a better fit. This is demonstrated in Figure 4.

5

Table 2.

Product & Label	In vivo PPD, JCIA	In vitro Prediction Based on:	
		UVA1&UVA2	UVA1
CTFA "A" SPF 17.27	3.21	4.15	3.48
CTFA "E" SPF 8.4	1.65	1.61	1.38
CTFA "F" SPF 9.5	3.09	3.9	3.28
CTFA "G" SPF 13.3	4.27	7.04	6.4
CTFA "H" SPF 5	3.8	4.4	4.25
CTFA "I" SPF 13.9	2.23	4.12	3.64
CTFA "J" SPF 30.7	12.43	15.9	14.16

Overall, it appears that this method is equally applicable to the

10   sunscreens with low, medium and high PFA (PPD) values containing wide

range of actives.

In vitro prediction of *in vivo* UVA protection provided by sunscreens

according to the present invention can be successfully utilized as a

15   preliminary step before PFA (PPD, JCIA) tests, which will provide excellent

in vitro/in vivo correlation; save time and resources; serve as an

optimization tool for sunscreen development and evaluation; and for

benchmarking.

20   A similar approach to that of the present invention may be applied

for *in vitro* determination of a sunscreens' protection potential against other

13

types of UV damage by utilizing various action spectra, irradiation spectrum of the specific light source and integration areas that are relevant to the test conditions.

5   It should be understood that the foregoing description is only illustrative of the present invention. Various alternatives and modifications can be devised by those skilled in the art without departing from the invention. Accordingly, the present invention is intended to embrace all such alternatives, modifications and variances.